## WHAT I CLAIM IS:

- 1. An insulin regulator construct, comprising:
  - a) a glucose response element (GIRE) of a liver-pyruvate (L-PK) gene promoter; and
  - b) an insulin-sensitive element of an insulin-like growth factorbinding protein-1 (IGFBP-1) basal promoter.
- 2. The insulin regulator construct of Claim 1, wherein:

said glucose response element comprises a hepatic nuclear factor-4 (HNF-4) binding site and a glucose responsive site.

- The insulin regulator construct of Claim 2, further comprising:
   a plurality of said glucose response elements.
- 4. The insulin regulator construct of Claim 2, wherein:

the sequence of said HNF-4 binding site and said glucose responsive site is in a native orientation.

5. The insulin regulator construct of Claim 2, wherein:

the sequence of said HNF-4 binding site and said glucose responsive site is reversed from a native orientation.

6. The insulin regulator construct of Claim 1, wherein:

said glucose response element is inserted upstream of said insulin-sensitive element in an insulin-like growth factor binding protein-1 (IGFBP-1) basal promoter.

7. The insulin regulator construct of Claim 1, wherein:

said glucose response element comprises a nucleotide sequence set forth in SEQ ID NO.: 1.

8. The insulin regulator construct of Claim 1, wherein:

said insulin-sensitive element comprises a nucleotide sequence set forth in SEQ ID NO.: 2.

9. An insulin regulator construct, comprising:

a nucleotide sequence set forth in one of SEQ ID NO.: 3, SEQ ID NO.: 4, SEQ ID NO.: 5, and SEQ ID NO.: 6.

 The insulin regulator construct of Claim 1, which is not stimulated by exposure to lactate or fructose.

- 11. The insulin regulator construct of Claim 1, which is stimulated by exposure to glucose and inhibited by exposure to insulin.
- 12. A vector comprising the construct of Claim 1.
- 13. An adenoviral vector comprising the construct of Claim 1.
- 14. A transgene comprising the construct of Claim 1.
- 15. A pharmaceutical composition comprising the construct of Claim1 and a pharmaceutically acceptable carrier or diluent.
- 16. A pharmaceutically acceptable derivative of the construct of Claim 1.
- 17. A method of treating or preventing diabetic conditions in a subject by administering an effective amount of the construct of Claim 1.
- 18. A method of regulating insulin production in a subject by administering an effective amount of the construct of Claim 1.

- 19. A method of modulating hyperglycemia, while avoiding severe hypoglycemia, in a subject by administering an effective amount of the construct of Claim 1.
- 20. A method of increasing fat catabolism in a subject by administering an effective amount of the construct of Claim 1.
- 21. A method of reducing protein catabolism in a subject by administering an effective amount of the construct of Claim 1.

## REFERENCES

- 1. Eisenbarth GS. Type I diabetes mellitus: <u>A chronic autoimmune</u> disease. N Engl J Med 1986; 314:1360-1368.
- Falqui L, Martinenghi S, Severini GM, et al. Reversal of diabetes in mice by implantation of human fibroblasts genetically engineered to release mature human insulin. Human Gene Therapy 1999; 10:1753-1762.
- 3. Muzzin P, Eisensmith RC, Copeland KC, Woo SLC. <u>Hepatic insulin</u> gene expression as treatment for Type 1 diabetes mellitus in rats. Mol Endo 1997; 11:833-837.
- Gros L, Riu E, Montoliu L, Ontiveros M, Lebrigand L, Bosch F. <u>Insulin production</u> <u>by engineered muscle cells</u>. Human Gene Therapy 1999; 10:1207-1217.
- 5. Short DK, Okada S, Yamauchi K, Pessin JE. <u>Adenovirus-mediated</u> transfer of a modified human proinsulin gene reverses hyperglycemia in diabetic mice. American Journal of Physiology 1998; 275:E748-E756.
- 6. Rivera VM, Wang W, Wardwell S, et al. <u>Regulation of protein secretion</u> through controlled aggregation in the endoplasmic reticulum. Science 2000; 287:826-830.
- 7. Selden RF, Skoskiewicz MJ, Russell PS, Goodman HM. <u>Regulation of insulin-gene expression</u>. N Engl J Med 1987; 317:1067-1076.
- 8. Kolodka TM, Finegold M, Moss L, Woo SLC. <u>Gene therapy for diabetes</u>

  <u>mellitus in rats by hepatic expression of insulin</u>. Proc Natl Acad Sci USA

  1995; 92:3293-3297.
- 9. Tuch BE, Tabiin MT, Casamento FM, Simpson AM, Marshall GM.

  <u>Transplantation of genetically engineered insulin-producing hepatocytes</u>

- <u>into</u> <u>immunoincompetent mice</u>. Transplantation Proceedings 1998; 30:473.
- 10. Valera A, Fillat C, Costa C, et al. <u>Regulated expression of human insulin in the liver of transgenic mice corrects diabetic alterations</u>. FASEB J 1994; 8:440-447.
- 11. Kaneda Y, Iwai K, Uchida T. <u>Introduction and expression of the human insulin gene in adult rat liver</u>. Journal of Biological Chemistry 1989; 264:12126-12129.
- 12. Yamaguchi M, Kuzume M, Matusumoto T, et al. <u>Insulin gene transfer compensates pancreatic á-cell function in diabetic rats</u>. Transplantation Proceedings 1998; 30:2913.
- 13. Sugiyama A, Hattori S, Tanaka S, et al. <u>Defective adenoassociated viral-mediated transfection of insulin gene by direct injection into liver parenchyma decreases blood glucose of diabetic mice</u>. Hormone and Metabolic Research 1997; 29:599-603.
- 14. Abai A, Hobart P, Barnhart KM. <u>Insulin Delivery with Plasmid DNA.</u>

  <u>Human Gene Therapy 1999</u>; 10:2637-2649.
- 15. Lu D, Tamemoto H, Shibata H, Saito I, Takeuchi T. Regulatable production of insulin from primary-cultured hepatocytes: insulin production is up-regulated by glucagon and cAMP and down-regulated by insulin. Gene Therapy 1998; 5:888-895.
- 16. Gros L, Montoliu L, Riu E, Lebrigand L, Bosch F. <u>Regulated production</u> of mature insulin by non-b-cells. Human Gene Therapy 1997; 8:2249-2259.
- 17. Wanke IE, Wong NC. Specific problems facing gene therapy for insulindependent diabetes mellitus: glucose-regulated insulin secretion from hepatocytes. Proceeding of the Western Pharmacology Society 1997; 40:131-133.

- 18. Simpson AM, Marshall GM, Tuch BE, et al. <u>Gene therapy of diabetes:</u>
  <u>glucose-stimulated insulin secretion in a human hepatoma cell line</u>
  (HEP G2ins/g). Gene Therapy 1997; 4:1202-1215.
- Powell DR, Suwanichkul A, Cubbage M, Lee PDK. <u>Regulation of insulin-like growth factor binding protein-1 (IGFBP-1) protein levels, mRNA levels and promoter activity by insulin (IN) and IGF-1 in HepG2.</u>
   Endo Society 1990:280A.
- Powell DR, Suwanichkul A, Cubbage ML, DePaolis LA, Snuggs MB, Lee PDK. <u>Insulin inhibits transcription of the human gene for insulin-like</u> <u>growth factor-binding protein-1</u>. Journal of Biological Chemistry 1991; 266:18868-18876.
- 21. Powell DR, Suwanichkul A. <u>HNF1 activates transcription of the human</u> gene for insulin-like growth factor binding protein-1. DNA and Cell Biology 1993; 12:283-289.
- 22. Suwanichkul A, Cubbage ML, Powell DR. The promoter of the human gene for insulin-like growth factor binding protein-1. <u>Basal promoter activity in HEP G2 cells depends upon liver factor B1</u>. Journal of Biological Chemistry 1990; 265:21185-21193.
- Suwanichkul A, DePaolis LA, Lee PDK, Powell DR. <u>Identification of a promoter element which participates in cAMP-stimulated expression of human insulin-like growth factor-binding protein-1</u>. Journal of Biological Chemistry 1993; 268:9730-9736.
- 24. Suwanichkul A, Morris SL, Powell DR. <u>Identification of an insulin-responsive element in the promoter of the human gene for insulin-like growth factor binding protein-1</u>. Journal of Biological Chemistry 1993; 268:17063-17068.
- 25. Suwanichkul A, Allander SV, Morris SL, Powell DR. <u>Glucocorticoids and insulin regulate expression of the human gene for insulin-like growth</u>

- <u>factor-binding protein-1 through proximal promoter elements</u>. Journal of Biological Chemistry 1994; 269:30835-30841.
- 26. Hughes SD, Johnson JH, Quaade C, Newgard CB. <u>Engineering of glucose-stimulated insulin secretion and biosynthesis in non-islet cells</u>. 1992; 89:688-692.
- 27. Rencurel F, Waever G, Antoine B, et al. Requirement of glucose metabolism for regulation of glucose transporter type 2 (GLUT 2) gene expression in liver. Biochemical Journal 1996; 314:903-909.
- Villafuerte BC, Goldstein S, Murphy LJ, Phillips LS. <u>Nutrition and Somatomedin XXV. Regulation of insulin-like growth factor binding protein-1 in primary cultures of normal rat hepatocytes</u>. Diabetes 1991; 40:837-841.
- 29. Ooi GT, Tseng LY-H, Tran MQ, Rechler MM. <u>Insulin rapidly decreases</u> insulin-like growth factor-binding protein-1 gene transcription in streptozotocin-diabetic rats. Molecular Endocrinology 1992; 6:2219-2228.
- 30. Pao C-I, Farmer PK, Begovic S, Goldstein S, Wu G-J, Phillips LS.

  <u>Expression of hepatic insulin-like growth factor-I and insulin-like growth factor-binding protein-1 genes is transcriptionally regulated in streptozotocin-diabetic rats</u>. Molecular Endocrinology 1992; 6:969-977.
- 31. Suh D-S, Zhou Y, Ooi GT, Rechler MM. <u>Dexamethasone stimulation of rat insulin-like growth factor binding protein-1 (IGFBP-1) promoter activity involves the interaction of multiple transcription factors.</u>

  Progress in Growth Factor Research 1995; 6:131-140.
- 32. Cuif M-H, Cognet M, Boquet D, Tremp G, Kahn A, Vaulont S. <u>Elements responsible for hormonal control and tissue specificity of L-type pyruvate kinase gene expression in transgenic mice</u>. Molecular and Cellular Biology 1992; 12:4852-4861.

- 33. Cognet M, Lone YC, Vaulont S, Kahn A, Marie J. Structure of the rat L-type pyruvate kinase gene. J Mol Biol 1987; 196:11-25.
- 34. Bergot M-O, Diaz-Guerra M-JM, Puzenat N, Raymondjean M, Kahn A. Cis-regulation of the L-type pyruvate kinase gene promoter by glucose, insulin and cyclic AMP. Nucleic Acids Research 1992; 20:1871-1878.
- 35. Vaulont S, Munnich A, Decauz J-F, Kahn A. <u>Transcriptional and post-transcriptional regulation of L-type pyruvate kianse gene expression in rat liver</u>. Journal of Biological Chemistry 1986; 261:7621-7625.
- 36. Goswami R, Lacson R, Unterman T. <u>Identification of insulin and glucocorticoid response sequences in the rat IGF binding protein-1 (IGFBP-1) promoter</u>. Endocrine Society 1993; 1915B:529.
- 37. Shu D-S, Ooi GT, Lesniak MAS. <u>Inhibition of IGFBP-1 gene expression</u> by insulin and stimulation by dexamethasone, cyclic amp, and phorbol esters are mediated by different cis-acting elements in the rat IGFBP-1 promoter. Endocrine Society 1993; 1916B:529.
- 38. Bergot M-O, Diaz-Guerra M-JM, Puzenat N, Raymondjean M, Kahn A.

  <u>Cis -regulation of the L-type pyruvate kinase gene promoter by glucose, insulin and cyclic AMP</u>. Nucleic Acids Res 1992; 20:1871-1878.
- 39. Smeekens SP, Chan SJ, Steiner DF. <u>The biosynthesis and processing of neuroendocrine peptides: identification of proprotein convertases involved in intravesicular processing</u>. Progress in Brain Research 1992; 92:235-246.
- 40. Groskreutz DJ, Sliwkowski MX, Gorman CM. <u>Genetically engineered</u> proinsulin constitutively processed and secreted as mature, active insulin. Journal of Biological Chemistry 1994; 269:6241-6245.
- 41. Steiner DF, Smeekens SP, Ohagi S, Chan SJ. <u>The New Enzymology of Precursor Processing Endoproteases</u>. Journal of Biological Chemistry 1992; 267:23435-23438.

- 42. Simonson GD, Groskreutz DJ, Gorman CM, MacDonald MJ. <u>Synthesis</u> and processing of genetically modified human proinsulin by rat <u>myoblast primary cultures</u>. Human Gene Therapy 1996; 7:71-78.
- 43. Unger RH, Foster DW. Chapter 21. In: Wilson JD, Foster DW, Kronenberg HM, Williams RH, eds. <u>Williams Textbook of Endocrinology</u>. Vol. 9th. Philadelphia, London, Toronto, Montreal, Sydney: W.B Saunders Co., 1998:973-1059.
- 44. Robertson DG, Marino EM, Thule PM, Seneviratne CK, Murphy LJ. Insulin and glucocorticoids regulate IGFBP-1 expression via a common promoter region. Biochemical Biophysical Research Communication 1994; 200:226-232.
- 45. Goswami R, Lacson R, Yang E, Sam R, Unterman T. <u>Functional analysis of glucocorticoid and insulin response sequences in the rat insulin-like growth factor-binding protein-1 promoter</u>. Endocrinology 1994; 134:736-743.
- 46. Suh DS, Ooi GT, Rechler MM. <u>Identification of cis -elements</u> mediating the stimulation of rat insulin-like growth factor-binding protein-1 promoter activity by dexamethasone, cyclic adenosine 3',5'-monophosphate, and phorbol esters, and inhibition by insulin. Molecular Endocrinology 1994; 8:794-805.
- 47. Goldstein S, Sertich G, Levan KR, Phillips LS. <u>Nutrition and somatomedin. XIX. Molecular regulation of insulin-like growth factor-lin streptozotocin-diabetic rats</u>. Molecular Endocrinology 1988; 2:1093-1100.
- 48. Minematsu S, Watanabe M, Tsuchiya N, Amagaya S. <u>Diurnal variations</u> in blood chemical items in Sprague-Dawley rats. Experimental Animals 1995; 44:223-232.

- 49. Haughton CL, Dillehay DL, Phillips LS. <u>Insulin replacement therapy for the rat model of streptozotocin-induced diabetes mellitus</u>. Laboratory Animal Science 1999; 49:639-44.
- 50. Koopmans SJ, Sips HCM, Krans HMJ, Radder JK. <u>Pulsatile</u> intravenous insulin replacement in streptozotocin-diabetic rats is more efficient than continuous delivery:effects on glycaemic control, insulin-mediated glucose metabolism and lipolysis. Diabetologia 1996; 39:391-400.
- 51. Wang RN, Bouwens L, Kloeppel G. <u>Beta-cell proliferation in normal and streptozotocin-treated newborn rats: site, dynamics and capacity.</u>
  Diabetologia 1994; 37:1088-1096.
- 52. Like AA, Guberski DL, Butler L. <u>Influence of Environmental Viral Agents on Frequency and Tempo of Diabetes Mellitus in BB/Wor Rats</u>. Diabetes 1991; 40:259-262.
- 53. Seglen PO. Preparation of rat liver cells. III. <u>Enzymatic requirements</u> for tissue dispersion. Exp Cell Res 1973; 82:391-398.
- 54. Ginot F, Decaux J-F, Cognet M, et al. <u>Transfection of hepatic genes</u> into adult rat hepatocytes in primary culture and their tissue-specific expression. Eur J Biochem 1989; 180:289-294.
- 55. Baker A, Saltik M, Lehrmann H, et al. <u>Polyethylenimine (PEI) is a simple, inexpensive and effective reagent for condensing and linking plasmid DNA to adenovirus for gene delivery</u>. Gene Therapy 1997; 4:773-782.
- 56. Marriott D, Gillece-Castro B, Gorman CM. <u>Prohormone convertase-1</u> will process prorelaxin, a member of the insulin family of hormones.

  Molecular Endocrinology 1992; 6:1441-1450.
- 57. Mittereder N, March KL, Trapnell BC. <u>Evaluation of the concentration</u> and bioactivity of adenovirus vectors for gene therapy. Journal of Virology 1996; 70:7498-7509.